In Vitro Adhesion and Platelet Aggregation Properties of Bacteremia-Associated Lactobacilli

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Eight bacteremia-associated Lactobacillus strains were evaluated in vitro for the ability to adhere to human intestinal mucosa and to aggregate platelets. Adherence varied significantly among the strains, and platelet aggregation was induced by three strains. In conclusion, strong binding ability does not appear to be a prerequisite for the involvement of lactobacilli in bacteremia or to their ability to aggregate platelets.

A total of 3,317 blood culture isolates were collected in southern Finland (population of ~2.5 million) during the years 1989 to 1992. Of these blood cultures, eight (0.24%) were identified as positive for lactobacilli. Most patients with positive blood cultures had severe underlying diseases that predisposed them to bacteremic complications. Saxelin and coworkers (12) characterized each strain and showed that the bacteria were different from the commercial Lactobacillus strains or natural isolates from dairy products at the time of the study period (Table 1).

Adhesion to human intestinal surfaces is one of the main selection criteria for probiotic bacteria. Another important criterion is lack of platelet aggregation potential, as this has been suggested to be harmful for the host, potentially leading to endocarditis. Adhesion ensures maintenance in the gut and increases the chances for probiotic microorganisms to influence the host’s microbial balance and gastrointestinal immune system (2, 10, 11, 13). Adhesion to the intestinal mucosa is, however, also considered a prerequisite for pathogenic bacteria to cause infections (1, 4, 6, 9), and in recent years the good adhesive properties of probiotics have raised some concern.

In this study, we assessed the adhesion and platelet aggregation properties of the eight Lactobacillus strains previously isolated from blood cultures of patients. The probiotic strain Lactobacillus rhamnosus GG (ATCC 53103) was included for comparison because it is known to adhere well to human intestinal mucus (7), ileostomy glycoproteins (IGP) (14), and Caco-2 tissue culture cells (15). It also does not cause spontaneous aggregation of human platelets (8). The aim of this study was to test the hypothesis that adherent lactobacilli have greater potential to cause platelet aggregation and to evaluate whether the abilities to bind strongly to intestinal surface and to induce aggregation of platelets are common features among lactobacilli implicated in bacteremia.

All bacterial strains used in the study were obtained in cooperation with H. Rautelin (Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland) and M. Saxelin (Valio Ltd., Helsinki, Finland). The origins and detailed classification of the isolates are shown in Table 1. All strains were cultured for 18 to 22 h at 37°C in de Man-Rogosa-Sharpe broth. Tritiated thymidine ([methyl-1-2H]thymidine)-labeled bacterial suspensions were prepared, and the mucus and IGP adhesion assays were carried out as previously described (7). As we have previously shown (7) that the adhesion of some Lactobacillus strains is dependent of the age of the mucus donors, the adherence of the clinical isolates to mucus isolated from newborns, 2- and 6-month-old infants, and adults was tested. The mucus stocks and the IGP preparation, which was a generous gift from J. G. H. Ruseler-van Embden (Erasmus University, Rotterdam, The Netherlands), were the same as in our previous studies (7, 14). The adhesion to differentiated Caco-2 cells (ATCC HTB 37) at day 15 was performed as described above except that the bacterial suspensions were prepared to phosphate-buffered saline (10 mmol of phosphate liter−1 [pH 7.2]), the optical density at 600 nm was adjusted to 0.5 ± 0.1, and 250 μl of each suspension was used to cover the Caco-2 monolayer in each well (24-well tissue culture plates).

Results are presented as the averages from four to seven independent (triplicate) experiments for adhesion to mucus and IGP and from two independent (triplicate) experiments for adhesion to Caco-2 monolayer. The statistical significance (P < 0.05) of the differences in the adhesive abilities of different bacterial strains was evaluated by two-factor analysis of variance. Two-tailed t test was used to evaluate the statistical significance of the differences in the adherence of the clinical isolates in comparison to that of the reference strain L. rhamnosus GG. The platelet aggregation assay was performed as described by Harty et al. (5), and results of duplicate aggregations are given as mean values and standard deviations (SD).

The ability to bind to intestinal mucus, IGP, and Caco-2 cells varied significantly between the different strains (Table 2). In comparison to the well-adhering probiotic control strain L. rhamnosus GG, clinical isolates 5, 6, and 7 adhered significantly (6.0 to 13.6%, P < 0.05) better to mucus and IGP, isolates 6 and 7 adhered significantly (5.4 to 7.7%, P < 0.05) better to Caco-2 cells, isolates 1, 4, and 8 adhered significantly (12.5 to 27.0%, P < 0.05) less to mucus, isolates 1, 2, 3, 4, and 8 adhered significantly (5.4 to 27.8%, P < 0.05) less to IGP, and isolate 4 adhered significantly (9.8%, P < 0.005) less to
Caco-2 cells. Adherence comparable to that of the probiotic control strain \textit{L. rhamnosus} GG (\(P > 0.05\)) was shown by five of the eight tested strains in the Caco-2 cell model and by isolates 2 and 3 in the mucus model. Most (five of eight) of the strains appeared to bind better to the mucus pooled from adults than infants (data not shown), which may be due to the age specificity (all strains were isolated from adult patients) and the fact that the intestinal environment of infants is still developing and provides fewer sites for adhesion. Maturing appeared to have the most significant effect to the adhesion of the isolate 8, binding of which was significantly increased with increasing age: 5.7\% \pm 2.3\% of the applied bacteria adhered to mucus of newborns, 7.4\% \pm 4.0\% adhered to mucus of 2-month-old infants, 13.5\% \pm 4.9\% adhered to mucus of 6-month-old infants, and 19.3\% \pm 2.7\% adhered to mucus of adults.

The results indicate that for these cases of bacteremia, good adherence to the intestinal mucosa was a common property but not a universal prerequisite for systemic infection. This may be due to the severe underlying conditions of the patients, which make it likely that in most cases the bacteria lack the pathogenic properties required to cause bacteremia. However, it is also possible that the isolates grown under in vitro conditions did not express the adhesion properties they would have in vivo. Alternatively, the bacteria may express different surface characteristics in the intestine (a superficial mucosal location) compared to the blood (invasive infection). Furthermore, a crucial factor in assessment of the safety of probiotic and dietary lactobacilli is whether invasion of the bloodstream is likely to occur through different epithelial surfaces, or to survive in the blood and/or tissues.

\[\text{TABLE 1. Origin of clinical \textit{Lactobacillus} isolates and classification by 16S rRNA sequence and by carbohydrate fermentation patterns}\]

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Diagnosis</th>
<th>\textit{L. rhamnosus}</th>
<th>\textit{L. casei (L. rhamnosus)}</th>
<th>\textit{L. rhamnosus}</th>
<th>\textit{L. paracasei spp. paracasei}</th>
<th>\textit{L. rhamnosus}</th>
<th>\textit{L. paracasei spp. paracasei}</th>
<th>\textit{L. casei (L. curvatus)}</th>
<th>Not in \textit{L. casei}, \textit{L. paracasei}, or \textit{L. rhamnosus} cluster (\textit{L. fermentum})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infection of aortic aneurysm graft</td>
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<td>2</td>
<td>Infection of abdominal aortic graft</td>
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<td>3</td>
<td>Carcinoma with liver metastasis</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Not available (patient died)</td>
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<td></td>
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<tr>
<td>5</td>
<td>Fever of unknown origin, urinary complaints</td>
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<tr>
<td>6</td>
<td>Respiratory infection (kidney transplant recipient)</td>
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<td>7</td>
<td>Salpingitis</td>
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<tr>
<td>8</td>
<td>Pneumonia (liver transplant recipient)</td>
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\[\text{\textsuperscript{a} Characterization was done by Saxelin and coworkers (12) by the API 50 CHL method.}\]
\[\text{\textsuperscript{b} V1 and V2 region sequences; there was 99\% similarity within a cluster.}\]
\[\text{\textsuperscript{c} Interpreted according to APILAB Plus V 4.0 (bioMérieux, Marcy l’Etoile, France).}\]
\[\text{\textsuperscript{d} Equivocal identification (unacceptable or doubtful profile according to APILAB Plus V 4.0).}\]

\[\text{\textsuperscript{e} Unequivocal identification (good, very good, or excellent profile according to APILAB Plus V 4.0).}\]

As new strains are continuously introduced into the market, it is important to gain a greater understanding of the factors that may play a role in the rare invasion of the blood stream by lactobacilli, so that their safety can be properly assessed (10). These factors may include the ability to adhere to and translocate through different epithelial surfaces, or to survive in the blood and/or tissues.

\[\text{\textsuperscript{a} Expressed as the percentage of bacteria adhered relative to the amount of bacteria added to the substrata (\textit{SD} for \(n = 4\) to \(7\) in mucus and IGP experiments and \(n = 4\) to \(2\) in Caco-2 cell experiments). The platelet aggregation properties of each strain are presented as the mean aggregation percentage (\textit{SD} for \(n = 2\)) per lag period in minutes (\textit{SD} for \(n = 2\)). \textsuperscript{1} Significantly higher (\(P < 0.05\)) in comparison to the reference strain; \textsuperscript{2} significantly lower (\(P < 0.05\)) in comparison to the reference strain.}\]

\[\text{\textsuperscript{b} Mean aggregation percentage (\textit{SD} for \(n = 2\)) per lag period in minutes (\textit{SD} for \(n = 2\)). ND, reaction not detected during 25 min of incubation.}\]
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REFERENCES


